CXXVII. THE OXIDATION OF THE FATTY DIBASIC ACIDS AND OF LAEVULIC ACID BY HYDROGEN DIOXIDE IN PRESENCE OF A CUPRIC SALT.

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CONSIDERABLE quantities of the dibasic acids are formed in the laboratory when the higher fatty acids are oxidised by such reagents as alkaline permanganate or nitric acid; and oxalic, succinic, adipic and suberic acids have been identified. It is however generally accepted that *in vivo* the path of the oxidation of the fatty acids does not proceed by way of the dibasic acids. Verkade and Van der Lee [1934] have however recently shown that when decanoic or undecanoic acid was taken by mouth, the terminal methyl group was oxidised and the corresponding dibasic acid excreted in the urine. In the laboratory, oxidation of the higher fatty acids with hydrogen dioxide is the process which has been supposed most closely to resemble the normal process of oxidation in the body, and recent experiments [Smedley-MacLean and Pearce, 1934] on the oxidation of palmitic acid, in which this process was catalysed by the addition of a cupric salt, have shown that at 90° there is a rapid breakdown, the end-products being succinic, carbonic and the volatile fatty acids. We therefore investigated the oxidation of the dibasic acids with the same reagents. These acids were found to be more readily attacked than the monobasic acids, so that even at 60° no glutaric or adipic acid and only 10 % suberic acid remained unchanged after 1 hour's heating. When palmitic acid was oxidised by excess of hydrogen dioxide in the presence of copper, there was very little evidence of keto-oxidation: the main products were hydroxy-acids, and there was also evidence of a considerable amount of desaturation. With the dibasic acids, definite evidence was obtained of keto-oxidation: thus laevulic acid was formed from adipic acid, a small quantity of acetonylacetone from suberic acid, and acetone was isolated from the products of oxidation of glutaric acid. With suberic acid however there was definite evidence of hydroxylation, the α-hydroxy-derivative and a lactonic acid being isolated.

From all the acids investigated (laevulic, suberic, adipic and glutaric), succinic acid was isolated as an oxidation product, and as succinic acid is itself oxidised under the conditions of the experiment giving a considerable proportion of carbonic and other volatile acids, the succinic acid isolated in these oxidation experiments must represent only a proportion of that actually formed. No evidence of the formation of fumaric, malic or tartaric acid was obtained.

The method of oxidation was that described by Smedley-MacLean and Pearce [1934], the volatile products being separated by steam-distillation.

The proportion of dibasic acids oxidised by a quantity of hydrogen dioxide sufficient to give about 19 % more than the amount of oxygen necessary for the complete oxidation of the acid was considerably augmented by the addition of

Table I. Oxidation of certain dibasic acids and laevulic acid carried out for 1 hour at 60°.*

Acid oxidised	Weight of acid g.	Total vol. ml.	Acidity (normality)	No. ml. 6% H_2O_2	In absence of copper					In presence of copper						
					CO_2% exp.†	CO_2% distillation‡	Reducing volatile acid calc. as % formic	Remaining volatile acids calc. as % acetic	Ether extract g.	CO_2% exp.†	CO_2% distillation‡	Reducing volatile acid calc. as % formic	Remaining volatile acids calc. as % acetic	Ether extract g.	Acetone g.	Aldehyde calc. as acetic g.
Succinic	2·5	300	0·0142	96	3·0	1·9	1·6	4·6	2·2	10·6	2·0	2·6	6·8	2·2	—	—
	2·5	300	0·0142	96	1·3	2·1	0·9	22·5	1·7	9·5	4·0	5·0	18·4	1·3	—	—
	2·5	300	0·0140	204	—	—	—	—	—	29·4	13·9	8·1	19·8	>0·3	—	—
	2·5	515	0·0242	350	—	—	—	—	—	45·6	10·1	1·8	14·0	0·6	—	—
Glutaric	2·5	300	0·0142	122	0·6	0·5	0·1	3·5	2·1	26·2	4·8	4·7	16·7	>1·4	—	—
	2·5	515	0·0346	350	—	—	—	—	—	53·3	13·1	2·8	40·2	0·3	—	—
	2·5	515	0·0242	350	—	—	—	—	—	42·7	27·9 §35·9	6·2	48·0 §22·0	1·0	0·004	0·059
	2·5	515	0·0242	350	—	—	—	—	—	44·1	14·3 §20·4	5·9	46·3 §25·4	1·0	0·042	0·031
Adipic	2·5	300	0·0041	150	2·0	7·1	0·04	5·2	2·4	4·0	3·8	2·0	8·3	2·2	—	—
	2·5	300	0·0142	144	0·5	6·8	0·2	3·1	2·2	15·0	7·3	5·4	8·6	1·8	—	—
	2·5	515	0·0222	350	0·2	2·7	0·2	4·1	2·1	32·2	17·0	4·0	24·2	0·6	—	—
	2·5	515	0·0222	350	0·1	5·2	0·4	11·4	—	30·9	14·6	4·4	24·4	0·7	—	—
	2·5	515	0·0222	350	—	—	—	—	—	22·2	13·7	4·0	21·8	0·4	—	—
Suberic	2·5	515	0·0186	350	0·2	1·2	0·1	1·2	2·1	5·3	6·2	4·8	7·1	1·4	—	—
	2·5	515	0·0186	350	—	—	—	—	—	7·8	9·5	4·2	10·3	1·9	—	—
	2·5	515	0·0275	350	—	—	—	—	—	9·8	8·1 §8·8	7·6	17·7 §15·2	1·6	0·010	0·008
Azelaic	2·5	515	0·0242	350	—	—	—	—	—	8·7	7·1	3·7	22·1	1·5	0·015	0·020
	2·5	515	0·0242	350	—	—	—	—	—	8·8	4·3 §9·0	6·3	41·4 §24·3	1·8	0·017	0·010
Laevulic	2·5	515	0·0142	350	—	—	—	—	—	14·3	17·9	2·9	34·8	1·2	—	—
	2	515	0·0058	350	—	—	—	—	—	15·7	11·9	2·2	29·1	0·8	—	—

* Percentages given are % carbon of original acid converted into the product concerned.
† Carbon dioxide evolved during the 1 hour's oxidation.
‡ Carbon dioxide evolved during subsequent steam-distillation after destruction of H_2O_2.
§ The lower figures give corrected values, allowance having been made for CO_2 retained as carbonate in the neutralised volatile acid distillates.

a cupric salt to the mixture; at the same time the proportion converted into carbonic and the lower fatty acids was much increased, the effect being most striking in the case of glutaric acid.

It will be seen from the figures given in Table I for adipic acid that a large increase in the amount of the hydrogen dioxide in the reaction mixture produced very little further oxidation in the absence of the copper salt. This agrees with the observation made by Dakin [1908] on the oxidation of the monobasic saturated fatty acids. Taking the percentage of the original acid converted into CO_2 and volatile acids as the criterion of the extent of oxidation, in the presence of the cupric salt, glutaric and adipic acids were more readily oxidised than succinic acid, both with a slight and with a large excess of the dioxide: in the latter case glutaric acid was almost completely converted into carbonic and the lower volatile fatty acids. A certain amount of carbonic acid was always given off during the steam-distillation of the product, markedly so in the case of glutaric and laevulic acids, probably owing to the decomposition of some unstable intermediate substance. Very little formic acid was present. Acetone in small amount was detected in the neutral volatile distillate after oxidation of glutaric acid; since acetone is hardly attacked by hydrogen dioxide in the presence of a copper salt, it is probable that little of the glutaric acid had undergone β-keto-oxidation.

When adipic acid was treated with a large excess of the dioxide in the presence of copper, no unchanged adipic acid was detected in the ether extract, but from 10 to 13 % of the total carbon had been converted into succinic and laevulic acids in the proportion of roughly 9 : 1. The succinic acid was identified by a mixed melting-point determination and the laevulic acid by the melting-point of its dinitrophenylhydrazone (200–202°), and a mixed melting-point with that of the same compound prepared from pure laevulic acid and melting at 202–204°. The formation of laevulic from adipic acid would be explained by oxidation at either the β- or γ-carbon atom, since in the dibasic adipic acid the β- and γ-derivatives are identical.
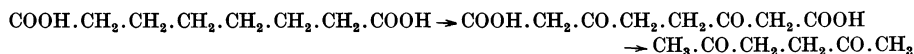
From the oxidation product of laevulic acid itself, carried out under similar conditions, small quantities of succinic acid and of acetone were obtained. Since on oxidation under these conditions, laevulic gave considerably less succinic acid than did adipic acid, laevulic acid was probably not the only intermediate substance formed. Succinic acid would also be formed by oxidation of the two α-carbon atoms, or possibly of the α- and δ-atoms, the α- and δ-positions in adipic acid being like the β- and γ-positions interchangeable. In the formation of succinic from glutaric acid, oxidation must have occurred at the α-atom (identical with the δ-position). The isolation of succinic acid from the higher dibasic acids in very much the same proportion is possibly due to a process of γ-oxidation by which a molecule of this acid is separated.

With a large excess of the dioxide, in the presence of a cupric salt at 60°, suberic and azelaic acids were less completely oxidised than the dibasic acids of lower molecular weight.

Under the above conditions from 20 to 40 % of suberic acid was converted into carbonic and the lower volatile fatty acids. The first two litres of the steam-distillate were neutralised and again distilled, the neutral distillate being collected as long as it gave a precipitate with dinitrophenylhydrazine hydrochloride solution. The first 200 ml. of this distillate were tested for acetaldehyde, and the subsequent distillate when treated with the reagent gave a hydrazone melting at 232–234°: this was very insoluble in most organic solvents but could be dissolved in glacial acetic acid and precipitated from this by water.

Microanalysis: (Found: C, 45·58; H, 3·72; N, 22·90 %. $C_{18}H_{18}N_8O_8$ requires C, 45·57; H, 3·80; N, 23·62 %). It was therefore identified as the di-dinitrophenylhydrazone of acetonylacetone: this substance was then prepared and the identity of the products established by determination of a mixed melting-point.

$\beta\beta$-Oxidation must therefore have taken place and acetonylacetone produced by elimination of CO_2 from the diketosuberic acid formed.

$$COOH.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.COOH \rightarrow COOH.CH_2.CO.CH_2.CH_2.CO.CH_2.COOH$$
$$\rightarrow CH_3.CO.CH_2.CH_2.CO.CH_3$$

From the glacial acetic mother-liquors, a very small amount of a more soluble hydrazone, melting unsharply at 100°, was obtained, but the quantity was too small for further investigation. In the preparation of the dinitrophenyl-hydrazone from acetonylacetone, after the separation of the di-dinitrophenyl-hydrazone melting at 245°, a second hydrazone melting at 167° was separated from the glacial acetic mother-liquors.

The products from four experiments in each of which 5 g. of suberic acid had been oxidised were worked up together and from these a total of 12·06 g. of an ether-soluble non-volatile acid product were obtained. This was neutralised with N NaOH, the equivalent of the acid being 86.

No residue was obtained from the ethereal extract of the neutral solution. After acidification with hydrochloric acid, the bulk of the acids were removed by ether extraction, the aqueous solution evaporated to dryness and the residue extracted first with ether and then with absolute alcohol.

The material soluble in ether was washed three times with chloroform, leaving 4·06 g. of an insoluble crystalline acid, giving both on titration and on analysis of the silver salt an equivalent of 71. By fractional crystallisation from water of similar material, two substances were separated: (1) less soluble crystals identified by a mixed melting-point with suberic acid, and (2) a readily soluble substance melting at 178° which did not depress the melting-point of pure succinic acid when added to it. The 4·06 g. of material insoluble in chloroform, which had an equivalent of 71, probably therefore consisted of approximately 2·38 g. succinic and 1·68 g. unchanged suberic acid. About 10 % of the original suberic acid had remained unoxidised.

The chloroform solution contained 4·84 g. of a yellow oil, of which 2·35 g. were soluble in benzene: the oily residue from the benzene solution on standing in an evacuated desiccator deposited colourless crystals of which 0·13 g., M.P. 109–111°, remained after draining on a porous tile. The equivalent of these determined by titration was 84 and by analysis of the silver salt 93. Similar crystals were obtained in another experiment but with an equivalent of 102 (calculated from the decomposition of the silver salt).

Analysis: (Found: C, 50·63; H, 6·60 %. $C_8H_{14}O_5$ requires C, 50·52; H, 7·37 %. Equivalent, 95. $C_8H_{12}O_5$ requires C, 51·06; H, 6·33 %. Equivalent, 94).

The solution of the sodium salt gave no precipitate with dinitrophenyl-hydrazine hydrochloride and did not decolorise potassium permanganate solution; the melting-point of a specimen of α-hydroxysuberic acid (M.P. 110–112°) did not appear to be appreciably depressed by the addition of a small amount of the crystals now isolated, so that in spite of the low hydrogen percentage these crystals may have consisted of α-hydroxysuberic acid.

The tile which had been used for draining these crystals was extracted with ether, and an oil sparingly soluble in light petroleum was obtained. Its equivalent determined by titration in the cold was 116: by warming with excess of $N/2$

NaOH and titrating the excess with $N/2$ H_2SO_4 the equivalent was found to be 85. Decomposition of the moderately soluble silver salt gave an equivalent of 86. The high equivalent obtained by titration in the cold suggested the presence of a certain amount of the lactone of a hydroxydibasic acid.

	% C	% H	Equivalent
Microanalysis: Found	49·57	6·96	86
Hydroxysuberic acid ($C_8H_{14}O_5$)	50·52	7·37	95
Lactone of hydroxysuberic acid	55·82	6·98	172
Hydroxypimelic acid ($C_7H_{12}O_5$)	47·73	6·82	88
Lactone of hydroxypimelic acid	53·16	6·38	158

A mixture of 2/3 hydroxypimelic and 1/3 of the corresponding lactone contains 49·54 % C, 6·67 % H and would have an equivalent of 111, which would diminish to 88 on opening the lactone ring. These figures agree fairly well with those actually found but we were unable to find any evidence of pimelic acid in the reduction product. Possibly the low carbon percentage was due to the admixture of succinic acid or other impurity with hydroxysuberic acid.

0·227 g. of the oil was boiled for 3 hours with 5 ml. acetic anhydride, 2·5 g. hydriodic acid (sp. gr. 1·7) and 0·25 g. red phosphorus. The mixture was cooled and diluted: a tarry oil separated and the whole was evaporated nearly to dryness under reduced pressure. The black residue was dissolved in alcohol, excess of phosphorus filtered off and the dark brown filtrate boiled for 1½ hours with 6 g. zinc dust and 10 g. glacial acetic acid. The filtered solution was concentrated to about 10 ml. and then considerably diluted, causing the separation of an oil, which was extracted by ether and saponified by boiling with 40 ml. 5 % alcoholic potash for 1 hour. After diluting the product with water, ether extraction yielded about 0·06 g. of a white crystalline neutral substance melting at 57–58° when recrystallised from benzene and alcohol. This was slightly soluble in water the solution giving traces of a precipitate with a solution of dinitrophenylhydrazine hydrochloride and a slight silver mirror with ammoniacal silver nitrate.

Microanalysis: (Found: C, 76·57; H, 12·86 %. $C_{10}H_{20}O$ requires C, 76·93; H, 12·82 %. $C_9H_{18}O$ requires C, 76·05; H, 12·68 %). No further evidence as to its constitution was obtained.

After acidifying the alkaline aqueous solution, ether extraction yielded about 0·12 g. of a white crystalline acid which after recrystallisation melted at 106–108° and dissolved in water to the extent of less than 1 %, thus resembling in its properties the α-hydroxysuberic acid, crystals of which had originally been separated from this oily product: pimelic acid melts at 103° and is about 5 % soluble in water; the melting-point of suberic acid is 139–140° and its solubility less than 1 %.

On the whole the evidence points to hydroxylation of the suberic acid having taken place in the α- and γ-positions.

From the chloroform solution 2·04 g. of an oil insoluble in benzene were obtained which after standing for some time in an evacuated desiccator deposited 0·64 g. colourless crystals. These were separated on a porous tile and recrystallised from benzene: they melted at 130–131° and their equivalent determined by titration in the cold was 102, by analysis of the white silver salt, which was comparatively stable to light, 99. A solution of the sodium salt gave no hydrazone when treated with a solution of dinitrophenylhydrazine hydrochloride and did not decolorise a solution of potassium permanganate, so that the presence of a saturated hydroxy-acid was inferred.

Microanalysis of the silver salt: (Found: Ag, 52·11; C, 24·22; H, 2·70 %. $C_8H_{12}O_5Ag_2$ requires Ag, 53·46; C, 23·76; H, 2·97 %).

The percentage of silver was estimated from the weight of the residue in the boat after combustion. The melting-point was considerably higher than that of α-hydroxysuberic acid and since there was no evidence of lactone formation, the presence of γ-hydroxysuberic acid could be excluded. β-Hydroxy-suberic acid, not hitherto described, may therefore have been present.

The oil from which these crystals had been separated contained 44·54 % C and 6·66 % H: the equivalent by titration was 98, by analysis of the silver salt 94: $C_7H_{12}O_6$ requires 43·75 % C; 6·25 % H; equivalent, 103. A dihydroxypimelic acid may therefore have formed the bulk of the oil.

On adding a solution of its sodium salt to a solution of dinitrophenylhydrazine hydrochloride, a very small amount of a precipitate, M.P. 85–90°, formed.

Microanalysis: (Found: C, 45·66; H, 4·01; N, 16·01 %. $C_{14}H_{16}O_8N_4$ (the dinitrophenylhydrazone of ketosuberic acid) requires C, 45·65; H, 4·35; N, 15·22 %); traces of a ketosuberic acid were therefore present in the oil.

The residue of the original oxidation product, which was insoluble in ether, was extracted with alcohol: most of this extract was soluble in water and consisted of a gummy substance which only very slowly reached constant weight and the nature of which was not determined.

The small amount of water-insoluble residue dissolved readily in alcohol and yielded a low-melting dark brown substance the constitution of which was not determined.

<div align="center">SUMMARY.</div>

1. Laevulic acid and the dibasic acids, succinic, glutaric, adipic, suberic and azelaic, were readily oxidised at 60° by hydrogen dioxide in the presence of a cupric salt, a considerable proportion being broken down to carbonic and the lower volatile fatty acids.

2. Succinic acid was identified as a product of oxidation of glutaric, adipic, suberic and laevulic acids.

3. Azelaic and suberic acids were less completely broken down than the lower acids.

4. From glutaric acid, a small amount of acetone was isolated, showing that β-keto-oxidation had taken place.

5. From both glutaric and laevulic acids, considerable amounts of CO_2 were obtained during the steam-distillation of the oxidation product, pointing to the presence of an unstable intermediate product.

6. From adipic acid, laevulic acid was obtained in appreciable amount, showing that β-keto-oxidation had taken place.

7. Only in the case of suberic acid was definite evidence forthcoming of the presence of hydroxy-derivatives. The evidence available pointed to the presence of α- and γ-hydroxysuberic acids and possibly of the β-hydroxy-derivative. The presence of hydroxy-derivatives of pimelic acid as intermediate products could not be excluded. The identification of a very small amount of acetonylacetone showed that some $\beta\beta$-keto-oxidation had taken place. The occurrence of a monoketosuberic acid and possibly of other keto-derivatives was also indicated.

<div align="center">REFERENCES.</div>

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